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Concentration - response functions for pentyl acetate have been established for the German shepherd and for human subjects tested in the same behavioral apparatus. In dogs the magnitude of performance decrement at a given concentration was found to be a function of the magnitude of the concentration reduction from the preceding test concentration if this reduction exceeded a certain quantity. In another series of experiments, data suggests facilitation of performance in dogs following ingestion of an odorant is not nonspecific but

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the degree of specification needs clarification. A third study concerned the measurements relevant to understanding the aerodynamics of the nasal chamber and the dispersal of odorant molecules in the nasal airways. The final study attempted to determine the minimum number of molecules necessary to excite a single olfactory receptor in the dog.

SUMMARY

Concentration - response functions for pentyl acetate have established for both german shepherds and human subjects tested in the same behavioral apparatus. The dynamic range of this function for the human subjects is in the order of <1 - 1 1/2 log units of concentration while that of the dogs is 3 - 4 log units. The absolute thresholds are in the range $10^{-5.0}$ - $10^{-5.5}$ of saturated vapor for human subjects and $10^{-6.5}$ - 10^{-8} for the dogs. The dog is thus about 10 - 1000 times more effective in detecting this compound than is man. This difference between canine and human subjects is less than that previously established for α-Ionone. The stimulus-response for pentyl acetate in both man and the best performing dogs shows a sharp notch comparable to that previously reported for α-ionone. It is centered at $10^{-4.5}$ in human subjects and at $10^{-6.0}$ - $10^{-6.5}$ for canine subjects. In the case of the dogs the magnitude of the performance decrement at a given concentration was found to be a function of the magnitude of the concentration reduction from the preceding test concentration if this reduction exceeded a certain quantity.

In an investigation of the influence of prior odorant ingestion on performance two dogs were trained to detect pentyl acetate (at a concentration of $10^{-6.5}$ of saturated vapor). One ml. of pentyl acetate was then administered to one dog and one ml. of propyl acetate to the other. Both dogs were then retested on $10^{-6.5}$ of pentyl acetate. With the exception of a transient initial depression in the performance of the second dog, both dogs improved their performance markedly to achieve a peak 8-9 days after ingestion. The improvement was greatest for the dog receiving pentyl acetate. Performances returned to within a standard deviation of the mean pretrial performance within 12-21 days after ingestion of acetates. In a further study one dog was retested on $10^{-6.5}$ pentyl acetate after ingestion of 1 ml of α -ionone. In the 10 days following the ingestion performance showed no significant deviation from the pretrial mean. This preliminary evidence suggests that facilitation of performance following ingestion of an odorant is not non-specific but the degree of specification remains to be clarified.

A third study concerned the measurements relevant to an understanding of the aerodynamics of the nasal chamber and the mode of dispersal of odorant molecules in the nasal airways. Quantitative analyses were made of duration; flow rate, and volume; of individual sniffs; position of peak sniff in a bout, and ratio of exhalation to inhalation during sniffing of three different odors (pentyl acetate; urine and anal gland secretions). Pentyl acetate was tested in different concentrations. The following conclusions were reached. (1) The dogs sniffing performance is not a stable, relatively invarient, response pattern (as has been claimed in other species), but varies for different odors and different concentrations of the same odor. (2) Unlike human sniffing the dog sniffs in a succession of alternating inspirations and expirations generally resulting in small net inhalation. (3) The most intense inhalation generally occurs at or near the end of a sniffing bout. It is usually twice the amplitude of preceding sniffs and four times the amplitude of succeeding sniffs (if any). (4) The mean duration of a sniff is about 100 m sec except for urine. (5) Lower concentrations of the same odor and more complex odors (e.g., urine) increase the number of sniffs in a bout. (6) The initial response to an odor is always an exhalation. (7) Urine elicited the most complex and distinct responses: The overall response was an exhalation (rather than an inhalation), and the mean sniff duration in a bout was about half that elicited by pentyl acetate. (8) Because of the small net changes in airflow during sniffing, it appears that the dog is moving a volume of odorous air backwards and forwards within the nasal chamber. This may relate to the need to create eddy currents which would carry

odorous molecules to the more remote recesses of the ethmoturbinal system.

A final study attempted to determine the minimum number of molecules necessary to excite a single olfactory receptor in the dog. The starting point was the absolute threshold for α-ionone established in a previous study. This value refers to odorant delivered to the dog's external nares. A study described below gives measurements of the mean volume of odorant inhaled by the dog in one sniff. Further estimates were made of odorant loss between the external nares and the olfactory surface (due to sorption of molecules on respiratory surfaces, etc.). Using this information calculations were made of the number of odorant molecules actually reaching olfactory receptor sites at threshold. This number is about 5×10^4 or about one molecule for every 4,000 receptors. Even allowing for an error of 10^{-3} this still implies that one receptor cannot detect a single molecule. The total area provided by the exposed surfaces of the plasma membrane of the dog's receptors is about 6.4 m or several times the area of the dog's body surface. Assuming a receptor site density comparable to that estimated for acetylcholine receptors in the neuromuscular junction, this would yield about 8×10^{16} olfactory receptor sites or about 4×10^{10} sites for every molecule of α-ionone present at threshold.

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PART - I. STIMULUS - RESPONSE FUNCTIONS FOR PENTYL ACETATE IN DOG AND MAN

Introduction

In previous reports we have described the results of studies which established stimulus-response functions for α-ionone in dogs and in human subjects. Among the salient findings was the appearance of two components in the stimulus-response functions raising the possibility that they reflect the action of two types of receptor site. The studies also showed differences of 1,000-10,000 in sensitivity to α-ionone between dog and man. It is conceivable however, that α-ionone is not typical in respect to those characteristics. Thus to give some insight into the generality of these findings (as well as to establish an appropriate concentration for testing dogs in the enhancement studies described in Part III) we have established stimulus-response functions of an odorant of quite different chemical structure - pentyl acetate, a straight chain aliphatic ester.

Methods

A full account of the methods used has been given in previous reports. What follows is a brief summary (see also Fig. 1).

Seven dogs were used, three being about 6 years old and four two years old at the beginning of the experiments. The odorant used was pentyl acetate (Eastman-Kodak).

Dogs were placed on a water deprivation schedule and trained in a programmed odor choice apparatus (Fig. 1) to sample each of three odor/air presentation bays. During trials odor is delivered to one bay, from an air-dilution olfactometer while air is delivered to the remaining two. The dog indicates the bay associated with the odor by sustaining the interruption of a photocell beam for five seconds. If the choice is correct the dog is rewarded with water delivered to a cup in the bay. If incorrect, access to the bay (and all others) is blocked by the lowering of a glass door. This seals off each bay and allows the next arrangement of odor and air (determined by the programmer) to reach equilibrium. After an intertrial interval of 30 seconds the dog can regain access to each bay by pressing on a treadle at the rear of the apparatus.

Three male human subjects were tested in the same apparatus under identical conditions with the following exceptions (1) To signal a response the subject interruped the photocell beam with his hand. (2) Instead of a water reward the subject received 10¢ for each correct response. (3) Since the human subject could not insert his head into the presentation bay, as could the dog, he used a teflon nose cone to sample from the perforated teflon odor/air diffusion plate at the base of each presentation bay.

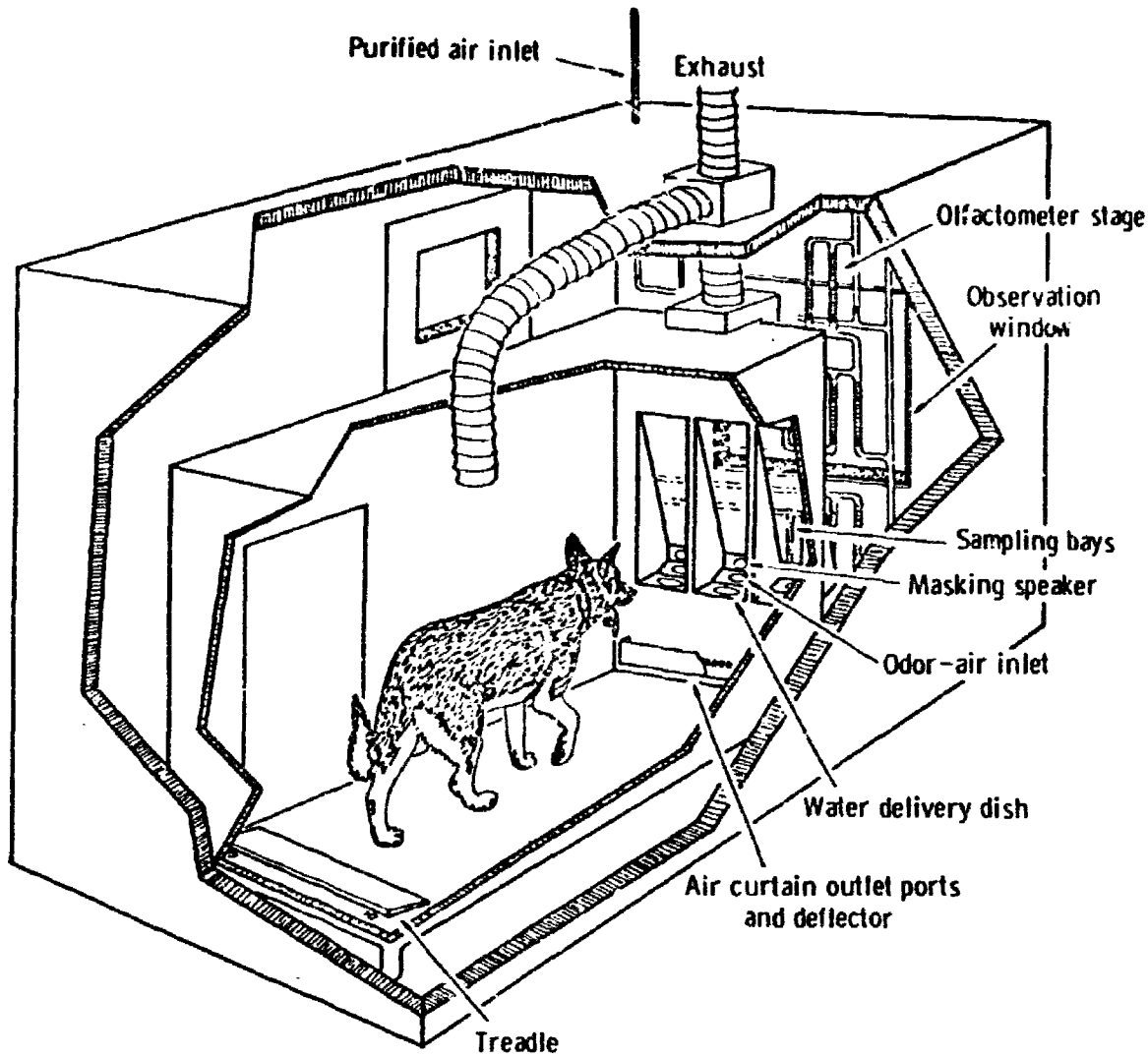

Fig. 1. Simplified view of controlled environment room containing test chamber. The height of the room has been reduced and certain details omitted for the purposes of illustration. (A gas chromatograph and water reservoir bottles normally rest on the roof of the chamber and an air conditioning unit and purification stages are housed on the roof of the room. The vapor saturator is not visible and the olfactometer is shown in simplified semi-schematic form.) The one-way glass windows normally reflect rather than transmit light from the angle shown here.

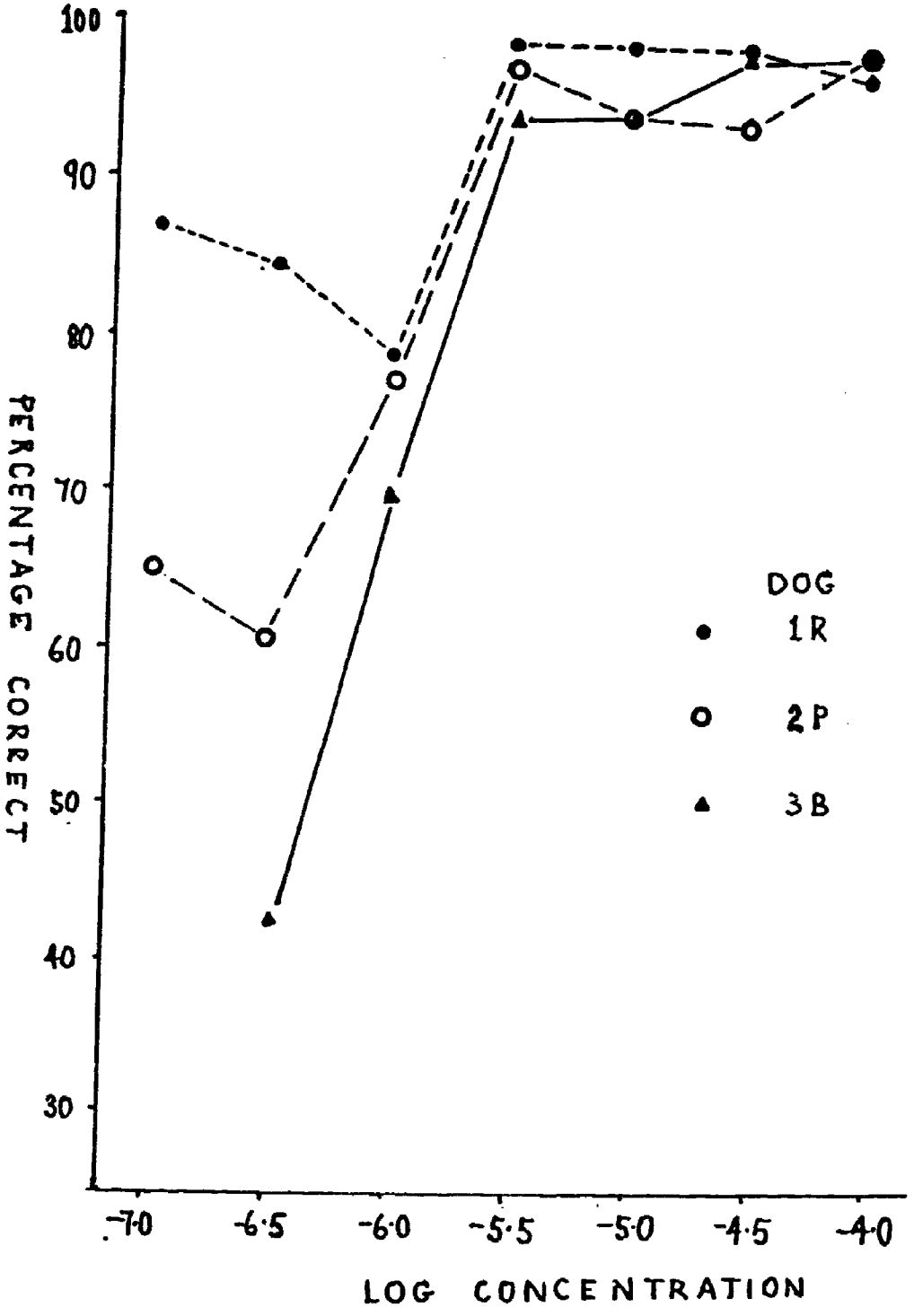

Fig. 2. Stimulus-response function for pentyl acetate in three older dogs.

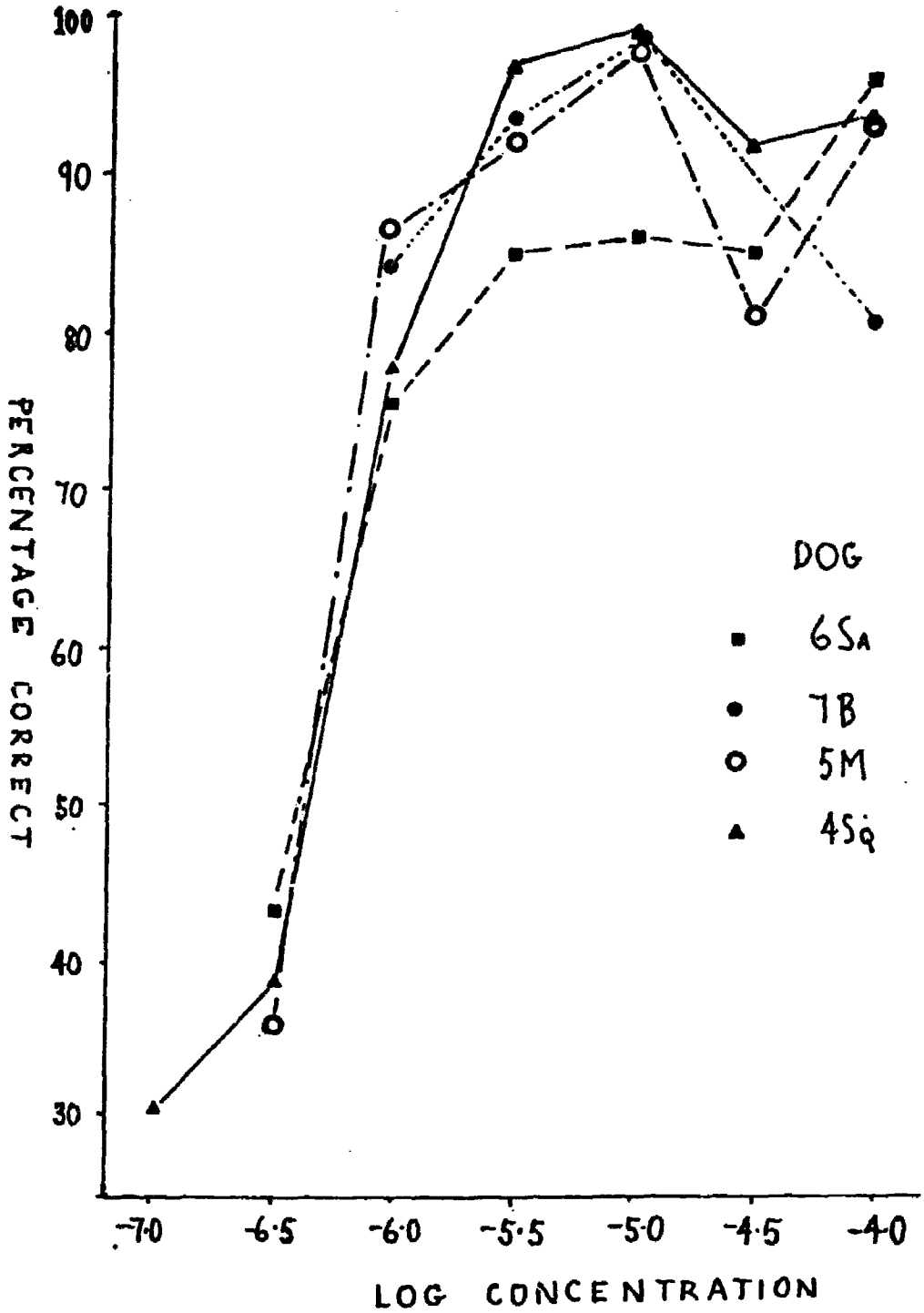

Fig. 3. Stimulus response functions for pentyl acetate in four younger dogs.

RESULTS

Stimulus-response functions for pentyl acetate are shown in Figs. 2 and 3. Data in Fig. 2 are incomplete (trials are still in progress). These data are for dogs about 6 years old. The data in Fig. 3, on the other hand are for younger dogs, about two years old. It is interesting that all the older dogs are superior performers at higher concentrations and with the exception of one dog (3B), maintain their superiority at lower concentrations. Among the poorest performers, however, is an old dog (3B). All dogs were tested over the same period. While the influence of age on performance is not clear, it is nevertheless apparent that younger dogs do not necessarily perform better than older dogs.

The break or reversal in the stimulus-response curve seen in the case of α-ionone is again apparent in the curves of two of the dogs tested here. Indeed the notch is more strongly developed for dog 1R than has previously been established for any dog in the α-ionone study.

Thresholds for all dogs whose curves are completed as well as for dog 2B lie in the range $10^{-6.5}$ - $10^{-7.0}$. For dogs 1R and 2P extrapolated thresholds lie in the range $10^{-7.5}$ - 10^{-8}.

The stimulus-response functions obtained with three human subjects are shown in Fig. 4. The baseline is the theoretical choice level (33%). As judged by these limited data human thresholds probably lie in the range $10^{-5.0}$ - $10^{-5.5}$ of saturated vapor. The sharp notch in the curves for two of the subjects is similar to that shown by two of the dogs (Fig. 2). The dynamic range is probably about 0.75 - 1.25 log units of concentration.

DISCUSSION

The similarity of the present data to those obtained on α-ionone is striking in several aspects . Firstly the clear notch on the curves is seen again although in a smaller proportion of the dogs. Again, the better the overall performance of the animal or human subject the more marked the notch tends to be with the poorest performers showing little evidence of it. However, the position of the notch in relation to the entire dynamic range of the curve is less constant than was the case with α-ionone.

The relative effectiveness of canine and human subjects shows a smaller gap than we previously found with α-ionone, the dog being 10 - 1000 times superior.

Data for pentyl acetate were obtained partly to allow selection of a concentration level for use in testing dogs in the experiments on altered detectability of odorants following their ingestion (see below).

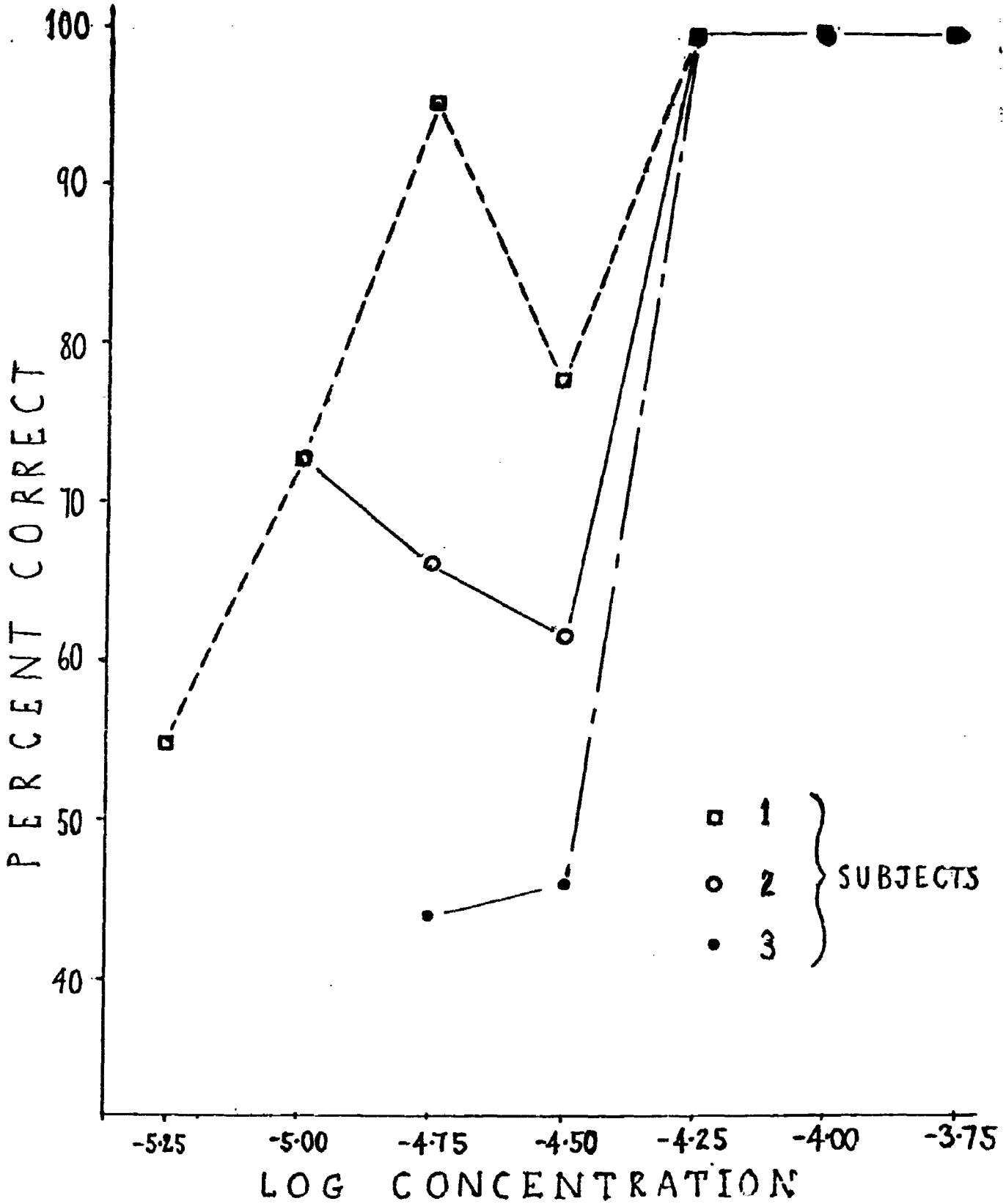

Fig. 4. Stimulus-response function for pentyl acetate in four human subjects.

PART II ENHANCEMENT OF RESPONSE TO ODORS

Introduction

We previously reported that α-ionone administered orally (1 ml) to a dog markedly altered the dog's ability to detect this odor when it was presented in the vapor phase. The next step has been to repeat this experiment using pentyl acetate, α-ionone and propyl acetate as the conditioning odorants and pentyl acetate as the test odorants.

Methods

On the basis of the stimulus-response curves derived for pentyl acetate in the previous section a concentration of $10^{-6.5}$ was selected to testing the performance of dogs 2P and 5M.

The mean performance levels for the dogs (±) S.E. were established in a series of control sessions. The experiment was initiated by oral administration of one 1 ml capsule of pentyl acetate to Dog 2P and of propyl acetate to Dog 6M. (In other words the 5 and 3 carbon atom members of the homologous series of saturated aliphatic acetates). The behavioral tests on pentyl acetate ($10^{-6.5}$) continued over the next 16 days for both Dogs and for 23 days for Dog 6M. In a further study, 1 ml of α-ionone was administered orally to Dog 2P and the dog then tested on pentyl acetate over the next ten days.

Results and Discussion

In the initial experiment, both dogs 2P and 6M showed marked departures from the mean score established before testing. (Fig. 5). In the case of Dog 2P the performance initially declined but rapidly rose on the second day to a score some 20 percentage points above the mean. Thereafter the performance declined and rose to a final peak (almost 30 percentage points about the mean score) on the eighth day before returning to within a standard deviation of the mean on the fifteenth day. Dog 5M (the dog receiving propyl acetate), also showed an overall tendency to increase its performance, reaching a peak on the 7th day before returning to baseline on the 20th day. However, the initial response after administration of propyl acetate was a decline in performance. Secondly, the difference between the mean and peak performances was only 23 percentage points.

In the final experiment (Fig. 6) Dog 2P showed no sustained trend in performance over the ten day period following ingestion of 1 ml α-ionone.

The striking feature of the results is the overall rise in performance of both dogs following ingestion of the acetates and the absence of any significant change following ingestion of α-ionone. To the extent that the enhancement of response tends to be less for propyl than pentyl acetate the effect may be related to the degree of similarity of the two compounds to the test odor - pentyl acetate. However, the differences are not sufficient to settle this question. It is to be expected that after the first few hours the compound ingested will have undergone metabolic alteration so that a related compound will be circulating in the blood. Thus if the observed enhancement is related to immunological sensitization or to induction of

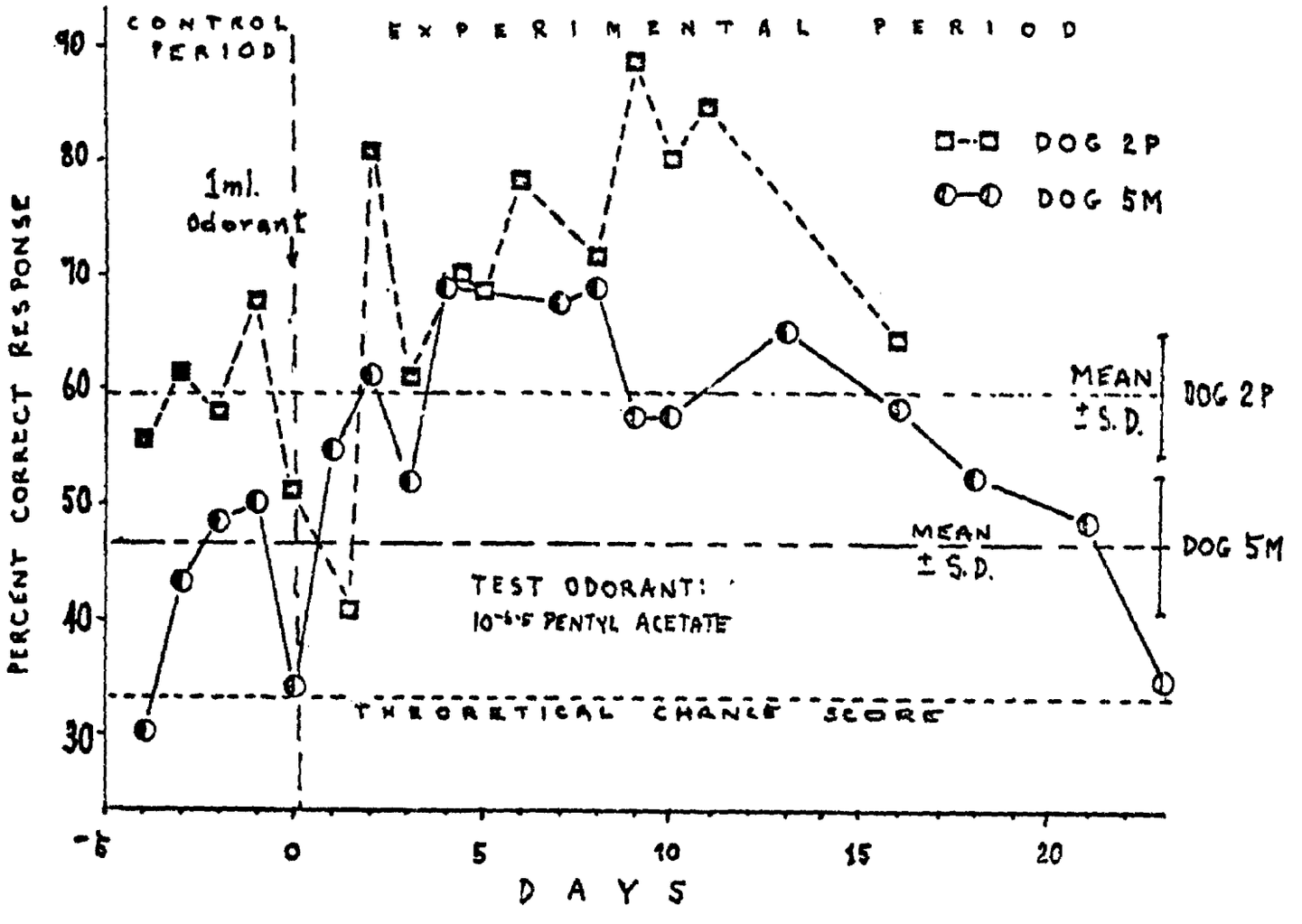

Fig. 5. Performance following ingestion of odor structurally unrelated to the test odor. Chance score is 33%. 1 ml α-ionone was administered at the point marked by the arrow Dog 2P.

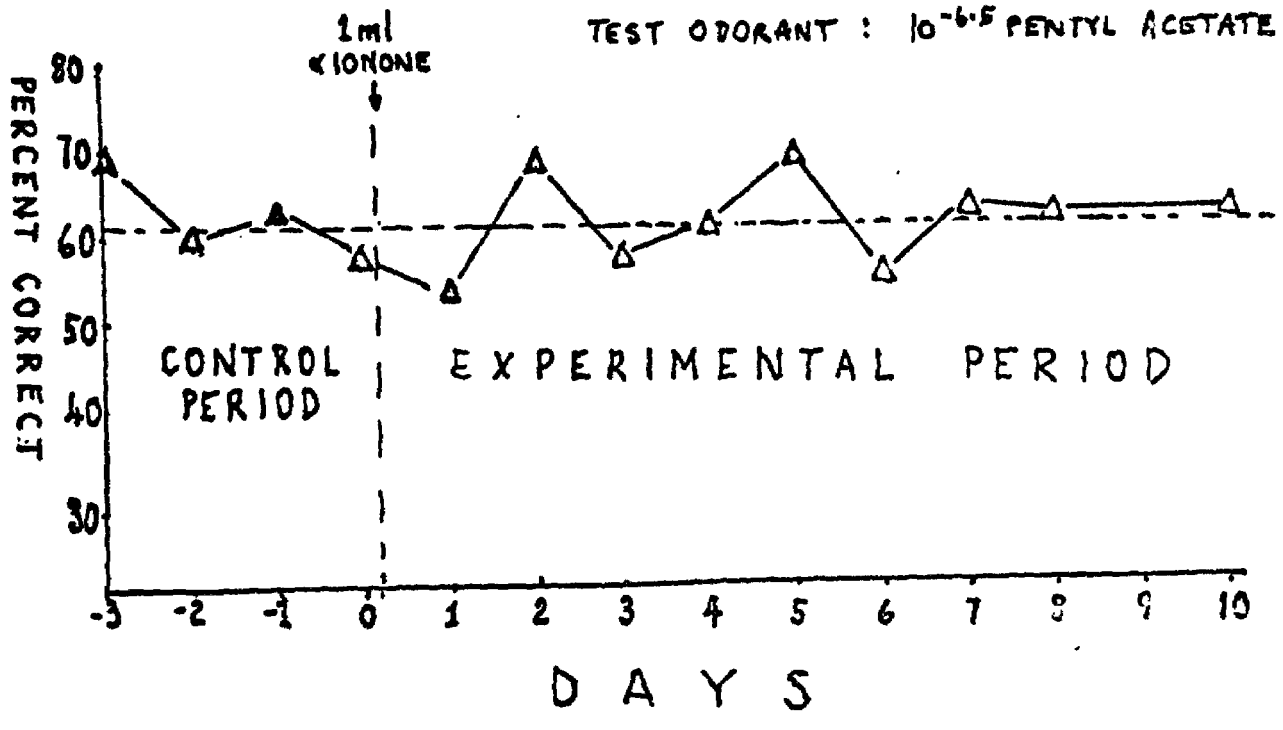

Fig. 6- Enhancement of response to odors following their ingestion.

receptor site formation by circulating volatiles it may be necessary to consider the breakdown products rather than the original odorant as the active agent. The failure of α-ionone to alter performance significantly is consistent with this possibility since it is so structurally distinct from propyl or pentyl acetate that this compound or any of its breakdown products are unlikely to induce the formation or sensitization of sites sensitive to the acetates.

While these are preliminary results they suggest that the enhancement of performance is not non-specific. However, the degree of specificity remains uncertain.

PART III. QUANTITATIVE ANALYSIS OF SNIFFING IN DOGS

Introduction

In earlier reports we outlined the rationale behind the recording analysis of sniffing in dogs performing an odor detection task. Briefly, the initial aim was to provide information about the number of molecules of odorant inhaled by the dog during a sniff and to determine whether there was any variation in sniffing pattern as a function of odorant concentration. The initial result with pentyl acetate showed that the sniffing bout (uninterrupted series of successive sniffs) was a more complex phenomenon in the dog than had been reported for other species, despite certain relatively constant features (for example, mean duration of an individual sniff was 100 - 110 m sec for two dogs). Among features contributing to variation in the internal structure of the sniffing bout is the position of the peak sniff in the bout and the amplitude and number of sniffs. Thus the mean flow rate of the maximum sniff in a bout increased linearly with increasing concentration of odorant. In addition, the mean number of sniffs in a bout was highest at the lowest concentrations tested, (which were near threshold).

Because of the unexpected variations found in the sniffing pattern it became clear that information was needed about sniffing patterns to other odorants particularly those with presumed biological significance for the dog (e.g. urine and anal gland secretions). In addition, it was necessary to investigate methods of analysing the data that would maximize the probability of detecting meaningful and consistent differences in sniffing patterns for different odors and for different concentrations of the same odor.

Methods

Subjects

Two female and one male German shepard were used. One female was about three years old at the start of the experiments while the other dogs were about one year old. They were housed in temperature controlled indoor runways, fed laboratory chow ad lib, and placed on a 23-hour water deprivation schedule. During testing and training they received an average of about 400 - 600 cc of water as rewards. The difference between this quantity and 1500 cc was given to them early each morning following the day of testing.

Behavioral test apparatus (Figs. 7 and 8)

The apparatus provides two bays, one associated with the odor of amyl acetate, and the other a blank. The two bays are set in a wooden console. Two swinging metal doors carry the sniffing ports. They are counterweighted to allow the dog to push them open but can be latched in position to block the dog's access to the water bowls (visible beneath the doors). The experimenter releases the latch by remote control when the dog makes a correct choice. The bowls are gravity fed from calibrated water reservoirs in the upper section of the console (Fig. 7).

Behind each sniffing port are two metal cylinders of similar length and diameter extended inwards by polyethylene cylinder. (Fig. 8). One of these is the Fleish pneumotachograph, the other is a dummy. To equalize flow resistance in the two cylinders the internal lumen of the dummy is fitted with a smaller cylinder. Since their relative positions can provide no differential cues, the cylinders occupy the same positions permanently. The cylinders are open at both ends but near the opening into the interior of the console there is a port on the floor of each polyethylene cylinder. This is made to accommodate a 10-cc vial, set so that its mouth is flush with the lumen of the cylinder. A loose glass wool plug about 3 cc in volume is placed into each vial. 25 drops of pentyl acetate in the diluent (ethylene glycol) are delivered to one vial and 25 drops of the diluent alone are delivered to the other. The sample vials are recharged after several runs are made to ensure reliable stimulus production. The relative positions of the vials (test and "blank") are varied according to a randomly determined sequence. Between trials each chamber was flushed out with a fan to ensure that no odor would remain to interfere with the next trial.

Each of the sniffing ports is surrounded with a ring of foam rubber. This allows the dog to insert its snout into the port without irritation yet seals tightly enough to prevent air leaking around the dog's snout.

Odorants

N-pentyl acetate was chosen as the first odorant because it has previously been used in olfactory studies (on rats, rabbits, tortoises, pigeons and man) involving both electrophysiological and behavioral apparatus; has a sharp distinctive odor with a known trigeminal threshold lying well above the olfactory threshold and has no known biological significance for the dog. It was diluted with ethylene glycol (Baker reagent grade) to the appropriate concentration. However, amyl acetate may have limited biological significance for the animal so anal sac secretion and male urine was also tested.

Since the odor in the cup is being diluted with the air drawn into the nosecone, the dog experiences a stimulus concentration considerably less than that present in the sample cup headspace. We earlier established that the dilution factor is about 1000:1.

To avoid any confusion stemming from these differences we will refer to concentration in solution as % concentration and concentration in air as a fraction of vapor concentration (10^{-3}, 10^{-4}, etc.) In the case of anal sac secretion and urine the raw sample was used without dilution.

Recording apparatus and its calibration

The output of the Fleish pneumotachograph was fed through a pressure transducer, amplified, monitored on an oscilloscope and stored on a tape recorder. Visual records of these tapes were later obtained from a multi-channel pen recorder. The pneumotachometer was calibrated by attaching a 1000-cc syringe and drawing air through it. The amplitude of the pen deflection was then plotted against flow rate. The response is linear over a wide flow range under the conditions of the experiment.

Training and Testing

Summarizing information given in earlier reports; dogs were first trained to indicate which door was associated with odor by pressing on it with their snout, and later by inserting their nose into the nose cone. No limits were set on the response time. Dogs were tested on a descending concentration series and responses to both odor and air were recorded. All concentrations, however, except the lowest, elicited performance scores well above the chance level.

Analysis of data

Initial analyses were directed towards identifying significant features in sniff patterns and determining how these differed (if at all) between air and odor for different concentrations of odors. Parameters chosen for study were: mean flow rate and volume of maximum sniff; mean duration of sniffs and of bouts; the number of sniffs in a bout the number of bouts in a train and position of peak sniff in bout. Sniff-volume was estimated by calculating the area of the equilateral triangle that best fitted the sniff trace. Since individual sniff records generally approximated a triangle the error involved is small.

Results

Performances on pentyl acetate (high and low concentrations) were compared with those on more complex odors (anal gland secretions and urine). The results are summarized in Tables 1 and 2 and Fig. 9.

Fig. 9 gives the averaged sequence of sniffs forming one bout. Even with direct visual inspection it is apparent that either increasing the complexity of the odor (anal gland secretion and urine) or lowering its concentration prolongs the sniffing bout. But the most prolonged and complex sniffing occurred in response to urine. Several features are shared in common with these bouts. (1) The most intense inhalation (downward deflection from baseline) occurs well into the bout and usually at the end or near it. This sniff is about twice the amplitude of preceding sniffs and almost always about four times the amplitude of successive sniffs. (2) The initial response is an exhalation. (3) The dog never sniffs in a series of successive inhalations followed by a prolonged exhalation (as seems to be the pattern in human subjects).

The conclusions following from inspection of Fig. 9 are confirmed and extended by the analyses summarized in Tables 1 and 2. Table 1 shows that the mean number of sniffs per bout as well as bout length almost doubles when a near threshold concentration of pentyl acetate is the test odor as opposed to a high concentration. The most prolonged bouts are in response to concentrated urine which elicits a mean sniff duration almost half that elicited by pentyl acetate. Urine is also unique in that the net airflow (total volume of air inhaled - air exhaled per bout) is a small exhalation as opposed to a marked inhalation for the remaining odors.

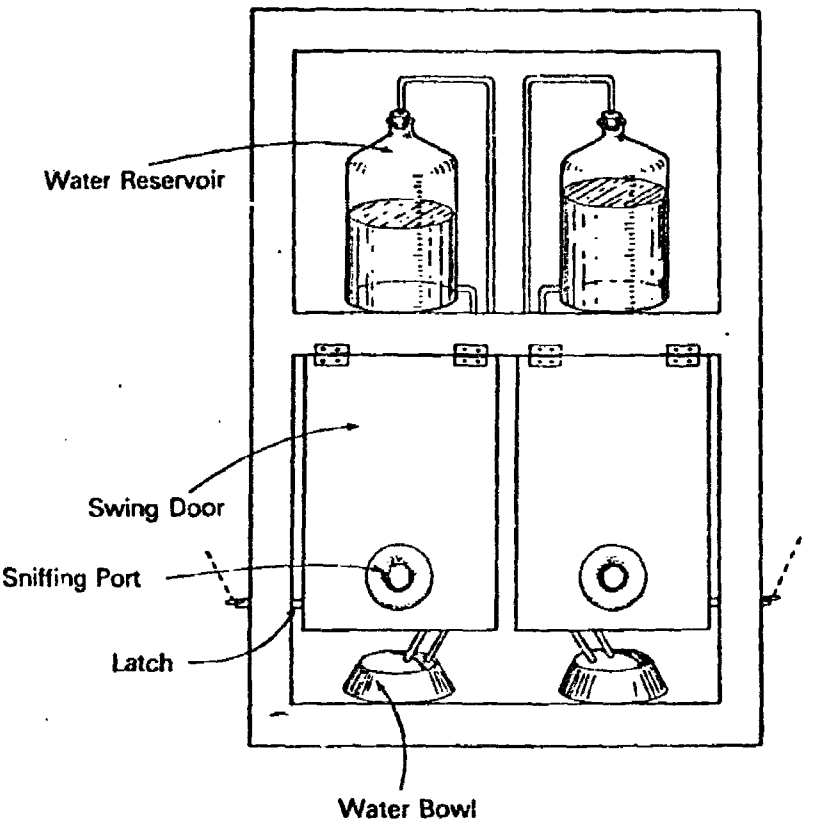

Fig. 7. Front view of odor discrimination console. Pneumotachograph lies behind sniffing port.

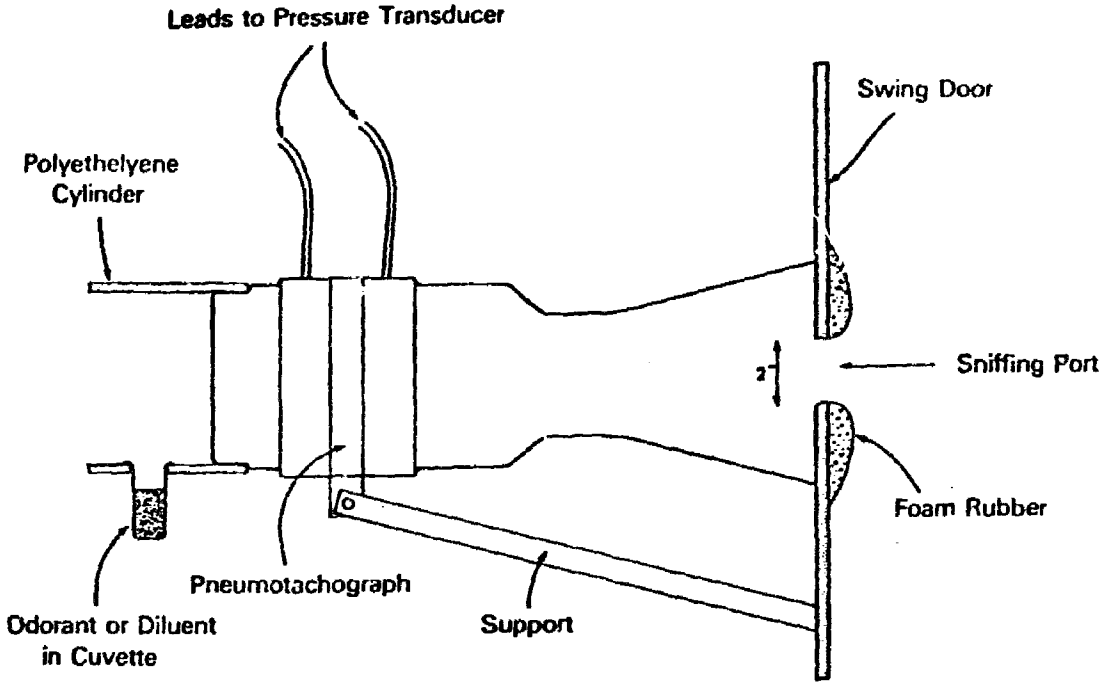

Fig. 8. Apparatus for quantitative measurement of sniffing in the dog. Side views of Fleish pneumotachograph attached to swing door. (Partially sectioned to show interior.)

The statistical significance of these differences is summarized in Table 3 where each test odorant is compared with every other odorant in turn for each of 7 parameters. Clearly urine stands out from the other 3 test odorants as differing significantly in the largest number of parameters - mean sniff length being the feature which differs in all cases. However, this is the only parameter which distinguishes responses to low concentrations of pentyl acetate from those to urine. Similarly response to low concentrations of pentyl acetate differs from response to anal gland secretions in only one parameter (position of peak sniff in bout).

DISCUSSION

Previous studies of sniffing behavior have been largely confined to the monkey and the rat. They have tended to emphasize that while changes in both duration of a bout and sniffing rates can occur under different behavioral conditions, sniffing is, in general, a stable and fixed response pattern. Such conclusions offer no preparation for the complex nature of sniffing behavior in the dog which the present studies reveal. Urine of a male dog having a complex odor with assumed biological significance for the female dog tested, clearly elicits a distinctive burst of sniffing which possesses its own internal structure and is distinct from all other odors tested in that the net air flow change is an exhalation rather than an inhalation. The dominance of low amplitude sniffing in a bout suggests that the animal may be shunting a small volume of odor backwards and forwards in its nasal chamber. This may favor the creation of eddy currents which could transport the odorous molecules into the more remote regions for the ethmoturbinals which bear the olfactory epithelium.

When a dog is working with either low concentrations of pentyl acetate or with urine odor the number of sniffs in a bout is larger than in the other conditions tested. This suggests that the dog sniffs more frequently with lower amplitude sniffs when it is dealing with a more difficult problem of detection or analysis, rather than increasing the amplitude of the sniff as man tends to do. However, human subjects tested on low concentrations of pentyl acetate or α-ionone did report that they tended to develop a pattern of low amplitude sniffs since this appeared to favor detection of the odor. But they did not alternate between sniffs and an exhalation as does the dog.

		Pentyl Acetate	Anal Gland	Urine
$\bar{X}$ sniffs per bout	P.H. High Conc.	--	.001*	.003*
	P.A. Low Conc.	.001*	.416	.143
	Anal Gland	--	--	.057
$\bar{X}$ sniff length sec.	P.A. High Conc.	--	.097	.004*
	P.A. Low Conc.	.262	.311	.016*
	Anal Gland	--	--	.008*
$\bar{X}$ bouts per trial	P.A. High Conc.	--	.080	.024*
	P.A. Low Conc.	.098	>.50	>.50
	Anal Gland	--	--	.184
$\bar{X}$ bout length sec.	P.A. High Conc.	--	.019*	.008*
	P.A. Low Conc.	.064	.416	>.50
	Anal Gland	--	--	.004*
Peak flow of peak sniff (L/min)	P.A. High Conc.	--	.0015*	.01*
	P.A. Low Conc.	<.002*	>.10	>.10
	Anal Gland	--	--	.016*
Position of peak sniff as ratio peak # to total # sniffs	P.A. High Conc.	--	.02*	.002*
	P.A. Low Conc.	.528	.052*	.206
	Anal Gland	--	--	.118
net airflow cc.	P.A. High Conc.	--	.07	.05*
	P.A. Low Conc.	>.10	>.10	.075
	Anal Gland	--	--	.02*

Table I. Probabilities (Mann-Whitney U-test) for paired comparisons between each of 4 solutions for each of seven parameters.
* Statistically significant differences.

	Pentyl Acetate High Conc. 1%	Pentyl Acetate Low Acetate	Anal Gland	Urine
$\bar{X}$ sniffs per bout	3.0	6.4	5.23	8.17
$\bar{X}$ sniff duration (sec.)	.11	.10	.095	.066
$\bar{X}$ bouts per trial	2.1	1.8	1.75	1.3
$\bar{X}$ bout length in sec.	.77	1.30	.99	1.48
Peak flow of peak sniff L/min.	72.5	40.83	49.07	42.86
Position of peak sniff as ratio: peak # to toal # sniffs	100%	85%	55.36%	71.75%
Net air flow cc.	+65.7(inhale)	+77.72(inhale)	+91.25(inhale)	-3.89(exhale)

Table II. Comparison of two concentrations of one odor and of two additional odors in terms of seven parameters related to sniffing. Data are from Dog 2P (female).

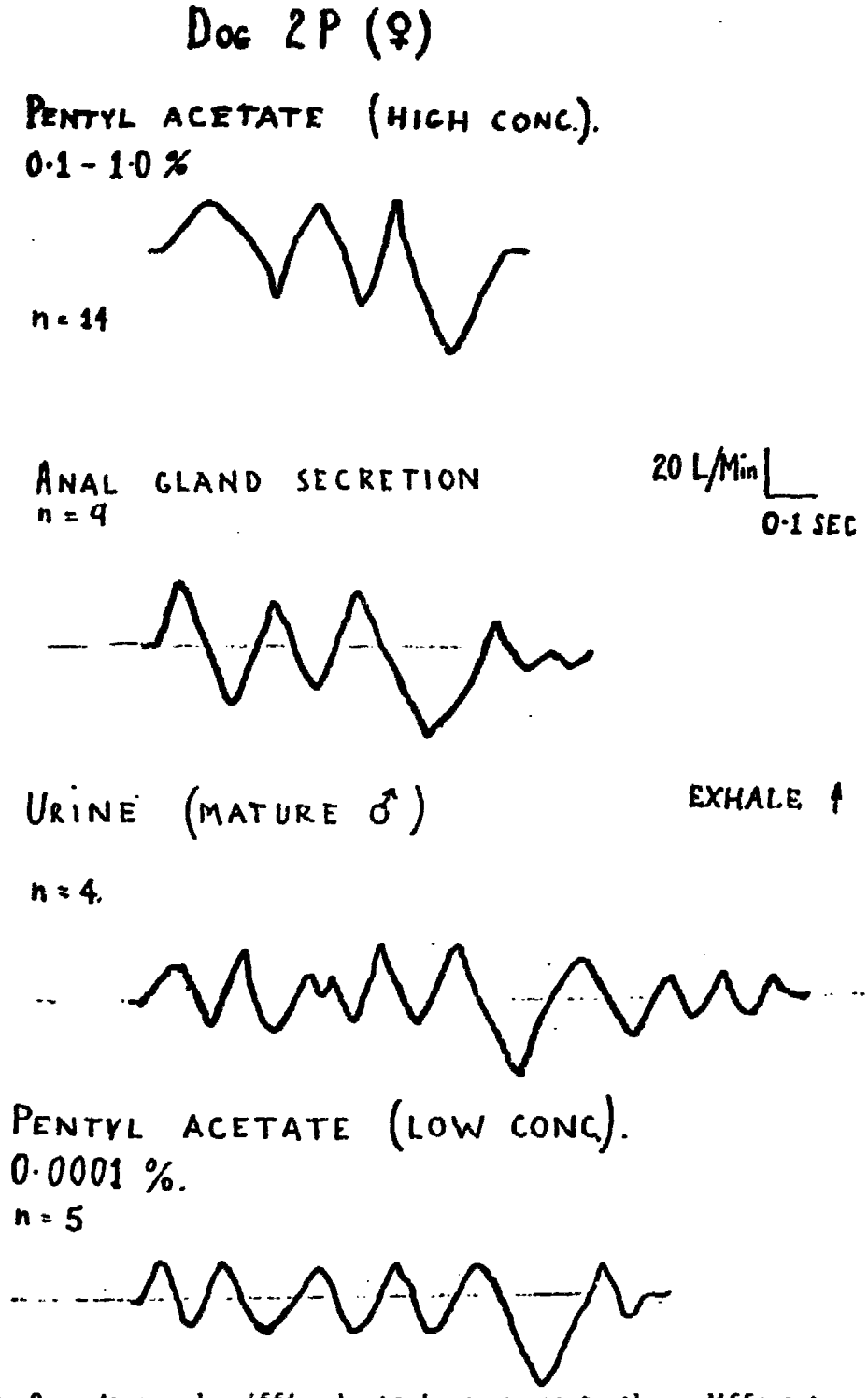

Fig. 9. - Averaged sniffing bouts in response to three different odors one of which (pentyl acetate) is presented in two different concentrations.

PART IV ABSOLUTE SENSITIVITY OF OLFACTORY RECEPTORS IN THE DOG

Introduction

Of the olfactory transduction process involves no more than a minute modification of ongoing events then the number of odorant molecules needed to excite the cell could be very small indeed. If, on the other hand, macrochemical events are involved we should expect much larger number of molecules to be involved. It therefore becomes important to estimate the mimimum number of molecules necessary to excite a single receptor. The closest we have come to this in the present series of studies has been to establish the number of molecules of α-ionone present per cm^3 of air sniffed by the dog at threshold. We cannot, however, move directly from this quantity to an estimate of the numbers of molecules necessary to excite a single receptor. The complexities of the nasal airways in the dog and the powerful sorptive properties of α-ionone, among other considerations, dictate that a series of calculations are necessary. One of these involves knowledge of the volume of air which a dog inhales in one sniff. As we have seen above, we now have an accurate measure of this quantity. Consequently, we are in a position to derive from the threshold of $4 \times 10^{4.5}$ molecules/cm^3 of α-ionone (obtained by the best performing dog) an estimate of the number that are necessary to excite a single receptor.

Equation For Estimating Number of Molecules Reaching Olfactory Receptor Sites

To determine the number of molecules reaching receptor sites we must know: (1) The minimum number of molecules of α-ionone that the dog can detect. (We can take this to be the amount contained in one sniff of a threshold concentration.) (2) The fraction (f_1) of inspired air that reaches the olfactory region. (3) The fraction of (f_2) of odorous molecules left in the air that reaches the olfactory region. (4) The fractions (f_3) of those molecules reaching the olfactory region which actually interact with receptor sites. (The remaining molecules escape sorption or bind to inactive sites.)

The number (N) of odorous molecules reaching olfactory receptor sites is then given by

$$N = N_0 f_1 f_2 f_3$$

To calculate N_0, assume the dog inspires about 60 cm^3 in one sniff of α-ionone. In the case of the best performing dog this represents about 5×10^6 molecules of the odorant at threshold. The magnitudes of the remaining fractions cannot be determined with precision. However, there are several lines of evidence on which we can draw to derive estimates.

(a) The Fraction of Inspired Air that Reaches the Olfactory Region

This fraction (f_1) depends on the course of the nasal air stream during active sniffing. Dr Vries and Stuiver (1960) estimates it to be up to 20 per cent for the human nasal cavity on the basis of a study of the flow of aluminum particles suspended in water in a model ofthe nose. However, there are wide variations among mammalian species in the pattern of nasal air flow. Thus in the dog (as in the rabbit, cat, rat and guinea pig), there si some evidence that a higher proportion of the total airflow courses along the floor of the nasal chamber -- away from the

olfactory region --than is the case in man (Lucas and Douglas, 1934; Becker and King, 1957). Assume, then, that f_1 is ten per cent.

(b) The Fraction of Odorous Molecules Left in the Air Reaching the Olfactory Region

This fraction (f_2) is also likely to be smaller in the dog than the 50 per cent estimated by dr Vries and Stuiver (1960) for man. The reason is the vastly greater sorptive surface presented to incoming air by the non-olfactory mucosa of the dog's nasal cavity. This includes the surfaces of a prominent swell body and complex maxilloturbinates -- structures absent in man.

Evidence bearing on this point comes from a study of the patterns of fluorescein sodium deposition in the nasal cavity of the dog. The dogs were induced to sniff the compound in the form of an aerosol spray. When the exposed nasal passages were viewed under ultraviolet light the heaviest concentrations appeared on non-olfactory surfaces (middle and inferior meatuses, oblique sulcus and nasopharynx). On the other hand, the ethmoturbinates, which support the olfactory epithelium, also fluoresced noticably (Becker and King, 1957). From the relevant figure in this publication, f_2 could be in the order or 30 per cent.

It might, of course, be argued that an aerosol of fluorescein would be unli ely to provide an adequate predictor of the pattern of deposition of α-ionone molecules at near-threshold concentrations. The evidence of Hornung et al. (1975) on frogs, suggests that, in fact, f_2 would be even smaller. They drew tritiated butanol through the nasal sac, froze the frog in liquid nitrogen and sectioned the nasal area. About 84 per cent of the molecules remained close to the external nares and less than 1 per cent reached the internal nares. While such high retentivity may not be characteristic of α-ionone, we can assume, as a first approximation, that f_2 is ten per cent.

The final fraction (f_3) is the proportion of molecules reaching the olfactory region that interact with olfactory receptor sites. This is particularly difficult to estimate since it depends on several factors, none of which are well understood. Firstly, there is difficulty of knowing whether a single odorant molecule interacts with only a single site, or whether it can interact with a number of sites in succession. Hornung and Mozell (1976), in an extension of the study described above (Hornung et al., 1973), found that a large proportion of the tritiated butanol molecules flowed over the olfactory sac, appeared to remain in the mucosa for at least 30 mins, and 90 per cent of those were deeper than 45 μm below the surface of the mucus. On the other hand, it is a common observation that the responses of single units in the olfactory epithelium to any one of a number of odorants decay to base line within about 500m. sec following cessation of the stimulus. Furthermore, the duration of a dog's sniff is probably about 100 m. sec. Within such time constraints the number of multiple "hits" that a single molecule can make may be limited.

A second difficulty is in knowing the extent to which binding to inactive sites occurs. Finally there is the problem of determining what proportion of molecules pass over the olfactory area but leave without coming into contact with the olfactory surface. If we assume that multiple hits compensate for failure of molecules to reach or to bind to active sites, f_3 becomes 1 and

$N = 5 \times 10^6 \times .1 \times .1 \times 1 = 5 \times 10^4$ molecules.

(c) The Ratio of Odorant Molecules to Receptors

Having estimated the number of molecular "hits" on active receptor sites we must now consider how many receptors are available. This requires knowledge of both receptor density and field size. It is not sufficient to know the extent of the olfactory area for an unspecified breed of dog since estimates for different breeds vary by more than a factor of 16. Fortunately, the area of the olfactory epithelium has been reported for the german shepherd: 169.46 cm^2, (Lauruschkus, 1942). On the other hand, receptor density for this breed is not known. It is, however, likely to fall within the range found for cat, guinea pig, rat and an unspecified breed of dog--namely, 50,000-215,000 receptors/mm^2. (See Altner and Kolnberger, 1975). Assume, then, that it is 120,000/mm^2. This implies that the german shepherd has about 2×10^9 receptors, which is about ten times more than has been estimated for the rabbit and 100 times more than has been estimated for man.

We cannot assume, however, that receptor sites are randomly distributed according to their odor specificities. There is evidence, derived electrophysiologically from the tiger salamander, that receptors sensitive to camphor, for example, are concentrated in the medial region of the ventral olfactory surface (Kauer and Moulton, 1974). Other anatomical and electrophysiological studies also suggest that there is a non-homogeneous distribution of receptor site types (see Moulton, 1976). However, some of these latter findings may at least partly reflect a further complicating factor, namely that odorant molecules are not necessarily distributed evenly across the olfactory surface. In particular, α-ionone is likely to bound relatively strongly. Consequently, the molecules will tend to concentrate on the more anterior surfaces. Regions lying remote from the respiratory air-stream within the recesses of the turbinal folds may receive no molecules. There is no data that would allow us to estimate accurately the consequenc of these features: the distribution of molecules or of sites for α-ionone molecules on the olfactory surface. As a first approximation, however, we can allow that their combined action may be comparable to restricting the number of available molecules to one tenth of the total olfactory surface.

On the basis of these calculations there are 2×10^8 receptors available to receive 5×10^4 molecules, or about 4,000 receptors to the molecule. Even allowing for an error of $+-10^3$ this still implies that one receptor can probably respond to one molecule of α-ionone.

(d) The Ratio of Odorant Molecules to Receptor Sites

In the context of the odorant-receptor interaction the significant unit is the receptor site, rather than the receptor itself. It seems probable that active binding sites are concentrated on the olfactory surface; the region of the receptor membrane in contact with the surface mucus. The most prominent structures at this level are cilia of which there are 100-150 per cell in the dog (Okano et al., 1967). Thus, if one takes the question of sensitivity to the level of the binding site it is necessary first to know the surface area (A) of the cilia and the density (D) of sites per unit area of cilia. From this one can calculate the total number of sites.

The first step in estimating A is determining the total surface area of a single cilium. Fortunately, its dimensions can be calculated, partly from data given by Okano et al. (1967) for the dog: From its base, a thickness of 0.25-0.3 μm, each ciliary shaft thins towards its distal end. Assume, then, a mean diameter of 0.2 μm. Ciliary length is uncertain but is probably comparable to that of the guinea pig: at least 50 μm (Calalano and Biondi, 1969). The surface area is, then, 31.42 μm^2 per cilium. Since there are about 125 cilia per cell and about 2×10^9 receptors in the german shepherd the total ciliary surface area is in the order of 7.85 m^2, or several times the area of the dog's body surface.

The second step is to determine D, the density of sites per unit membrane surface. This is not known. For the present purposes, however, another membrane system specialized to detect low concentrations of a chemical may be the closest model for which pertinent information exists. This is the subsynaptic membrane of the neuromuscular junction bearing sites receptive to acetylcholine. The density of the sites is calculated to be in the order of $10^4/\mu m^2$ (see Gage, 1976). Assuming this can also be taken as an estimate of A, the total number of olfactory receptive sites becomes 8×10^{16}. If only one tenth of these sites are accessible to α-ionone there are still about 4×10^{10} receptor sites available for each molecule of the odorant.

CONCLUSIONS

The conclusion that one molecule of a specified odorant is sufficient to excite a single olfactory receptor agrees with the estimates of both Nenhaus (1953) for the dog and de Vries and Stuiver (1960) for man. (This is despite the fact that the calculations are now based partly on much evidence unavailable to the earlier authors.) Apparently the dog has little or no advantage over man at the single receptor level. Furthermore, whatever may be the nature of the transduction process in olfaction, it would seen to require almost negligible energy changes to activate it.

Where the dog does differ from man, however, is in the receptor reserve available. Our calculations suggest that there are over a billion receptors in the olfactory epithelium of the german shepherd or somewhat more than 100 times the number given for man. This is reflected in a comparison between the ratio of receptors to odorant molecules available at threshold. It contrast to the ratio of 4,000:1 for the german shepherd it is closer to 1:1 for man (de Vries and Stuiver). This reserve may come into play in the detection of compounds having lower thresholds than α-ionone. It may also be critical in the spatial analysis of complex mixtures of odorants.

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